

Bacterial flora of farmed mud crab, *Scylla serrata* (Forsk., 1775) and farm environments in Kerala, India

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ABSTRACT

The microflora associated with mud crab *Scylla serrata* collected from farms located in Kerala, India were investigated. The microbial load and types of bacteria associated with farm water, sediment and crabs were monitored. The mean mesophilic count of $5.67 \log_{10} \text{cfu g}^{-1}$ in farmed fresh crab indicates acceptable crab quality. The levels of enterococci and faecal coliforms in farmed mud crab were high. The microbial groups, most commonly isolated from crab meat were *Vibrio*, Enterobacteriaceae, *Moraxella*, *Acinetobacter*, *Pseudomonas/Shewanella*, *Micrococcus* and *Bacillus*. The percentage composition of various genera in crab meat varied between farms. The study reports a diverse array of bacterial species, including several potential human pathogens such as *Vibrio fluvialis*, *Vibrio hollisae*, *Vibrio mimicus*, *Aeromonas veronii* biovar *sobria*, *Aeromonas caviae* and *Aeromonas jandaei* from edible crabs. Owing to the potential hazard of these pathogenic bacteria, it is necessary to put more emphasis on hygienic handling of farmed crab. This study confirms that farmed crabs carry significant numbers of motile aeromonads, capable of growth at low temperature. Immediate icing of harvested crabs is essential to improve the microbiological stability.

Keywords: Farmed mud crab, Commensal flora, Pathogenic bacteria, *Scylla serrata*, *Vibrio*

Introduction

Crab aquaculture has been identified as an emerging aquaculture sector with significant potential. Farmed crab production in 2005 reached 660,000 t globally, with virtually 100% contribution from Asia (Paterson, 2009). Mud crabs belonging to the genus *Scylla* are large portunids with high commercial value. In India, mud crabs have come into prominence, since early eighties, with the commencement of live crab export to the south-east Asian countries and this has created a renewed interest in the exploitation as well as in the production of mud crabs through aquaculture. In view of the widespread disease problems in shrimp farming during 1990s, farmers started looking for alternate, more disease resistant and economically important commercial fish species. Live mud crabs (*Scylla serrata* and *Scylla tranquebarica*) being a much sought after export commodity, the farming of mud crabs emerged as the best alternative. Commercial activity in crab farming increased in India which is based primarily on capture and fattening of juvenile crabs from the wild. Cannibalism is the greatest constraint to productivity in all the communal growing systems. About 11 types of crab products are being exported from India, pinpointing its importance in the foreign exchange earnings. The high value of crab meat and its susceptibility to spoilage have

promoted investigations, into the microbial flora and organisms responsible for spoilage (Cockey and Chai, 1991). One of the main products currently being pursued commercially is soft-shell crab which is becoming popular worldwide and fetches high value.

The environment has great influence on the bacterial flora of freshly caught crabs. During processing, including peeling and picking, bacteria present on the body surface or in the intestine can be introduced into the crab meat and cause contamination. Lee and Pfeifer (1975) studied the microbiological characteristics of dungeness crab (*Cancer magister*) processed and retailed in Oregon, USA and they reported that the growth of psychrotrophic microorganisms of *Pseudomonas*, *Moraxella* and *Acinetobacter* spp. during refrigerated storage was the major contributing factor for the high microbial count of the retail crab meat. Bacteria associated with spoilage of canned, pasteurized crab cake mix product stored at various temperatures have been reported (Loaharanu and Lopez, 1970). In spite of the increase in aquaculture production of mud crabs and their value for export, relatively little attention has been given to the microbiological quality of crab and quality changes during post-harvest handling. The objective of this study was to identify the commensal and pathogenic bacteria associated with farmed mud crab *S. serrata* as well as from the farm environment.

Materials and methods

Collection of sample

The mud crab, *S. serrata* were collected from four farms located in Kerala (southern India). The type and location of the crab farms and species farmed are shown in Table 1. Water samples were collected from different locations in each farm by inverting sterile 1 l polystyrene bottles into the water to about 30-40 cm below the surface. Farm sediment samples were scooped out from different locations of the farms, and collected in sterile polyethene bags. Farm water and sediment samples, collected from different locations were packed in polythene pouches and transported on ice to the laboratory. Samples were pooled before analysis and tests were initiated within 2-3 h (Lalitha and Surendran, 2004). Crabs, farm water and sediment from each farm were sampled twice in a year in 2004 and 2005. Crab samples (12-15 nos.) were packed in sterile polythene pouches, transported live on ice in ice box (10-12 °C) and aseptically analyzed within 2-4 h after being caught. On arrival at the laboratory, raw crab meat was separated from the shell aseptically. Claw meat was also analysed.

Physico-chemical parameters of farm water

The hydrographic parameters *viz.*, pH, temperature and salinity of farm water were analysed according to standard methods (APHA, 1998).

Microbiological analyses

Samples of raw crab meat or claw meat (25 g) were aseptically taken and transferred to a stomacher bag (Seward Medical, London, UK), 225 ml of physiological saline (Na Cl, 0.85% w/v) was added, and the mixture was homogenized for 60 s with a stomacher (Lab blender 400, Seward Medical, Norfolk, IP24, IXB, UK). Samples (1 ml) of serial dilutions of crab homogenates were plated on Tryptone Soya Agar (TSA Oxoid, UK), incubated at 37 °C and 30 °C for 2 d for determination of total aerobic mesophilic counts, at 20 °C for 4 d for total aerobic counts and at 7 °C for 10 d for determination of psychrotrophic counts. Three replicates of at least three appropriate dilutions were enumerated. *Staphylococcus aureus* counts were estimated on Baird Parker Agar (BP, Oxoid U.K) at 37 °C for 2 d and typical colonies were confirmed by coagulase test (FDA, 1998). Percentages of confirmed

colonies were used to correct the results of the counts obtained. Total *Vibrio* counts were performed by surface plating on Thiosulphate Citrate Bile Sucrose Agar (TCBS, Oxoid) incubated at 37 °C for 24 h (Bolinches *et al.*, 1988; Maugeri *et al.*, 2000; Pfeffer and Oliver, 2003). Twelve to fifteen numbers of both sucrose fermenting and sucrose non-fermenting colonies, distinguished by their yellow and blue-green colours, respectively, were picked for characterization and identification using standard biochemical tests (Barrow and Feltham, 1993; Elliot *et al.*, 1998) and biochemical keys (Alsina and Blanch, 1994). The identification of the isolates was also confirmed by API-20E system (Bio Merieux, France).

Coliforms, faecal coliforms and *Escherichia coli* counts were determined by a 3 replicate tube MPN (Most Probable Number) procedure (APHA, 1998). Positive EC tubes were confirmed for *E. coli* by streaking on to Eosine Methylene Blue Agar (EMB Agar, Difco, Detroit, Michigan) and for identification of the characteristic colonies (APHA, 1998). Enterococci numbers were estimated on KF Streptococcal Agar (Difco, Detroit, MI) and characteristic colonies were confirmed by biochemical tests as per APHA (1998). *Clostridium perfringens* numbers were determined by the three tube MPN method using Lactose Sulphite Broth (West, 1989) and confirmed by streaking on to Tryptose Sulfite Cycloserine (TSC) agar and identification of characteristic colonies (FDA, 1998).

Bacterial numbers in cfu g⁻¹ or MPN g⁻¹ sample were transformed into log₁₀. Statistical analyses of the bacterial parameters were performed using the statistical tool package of Microsoft Excel 97 software. Student's t- test was used to evaluate the significance of differences between means of microbial counts in water, sediment and crab samples at 37, 30, 20 and 7 °C.

Isolation and identification of bacteria

A total of 286 bacterial cultures were randomly selected and isolated from TSA plates (30 °C) sampled from whole crab and water from four farms. All colonies from a sector of the plate or all colonies from a whole plate were isolated, purified and stored on TSA slants. They were characterized morphologically and biochemically. The strains were tested for gram reaction, catalase and oxidase

Table 1. Details of the crab farms selected for the study

Farm	Monoculture / polyculture	Area of pond (acre)	Species cultured
1	P	0.5	<i>Scylla serrata</i> , <i>Chanos chanos</i> , <i>Penaeus indicus</i>
2	P	1	<i>Scylla serrata</i> , <i>Chanos chanos</i> , <i>Penaeus monodon</i>
3	P	1	<i>Scylla serrata</i> , <i>Penaeus indicus</i>
4	M	0.8	<i>Scylla serrata</i>

reactions, motility, oxidative/fermentative metabolism and presence of spores. They were then grouped according to the taxonomic schemes of Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984; Sneath *et al.*, 1986), further tested for the most relevant characteristics of each group and identified, using the schemes proposed by several authors for identification (Krieg and Holt, 1984; Sneath *et al.*, 1986; Austin, 1988; Kirov, 1997).

Results and discussion

Physico-chemical characteristics of water

The hydrographic parameters, temperature and pH values, of water were similar in all the four farms and are within the acceptable range for rearing mud crab (Table 2). Temperature ranged between 29-31 °C and pH values were between 6.0 and 7.0. The lowest salinity (ppt) levels (4.0 to 5.0) were recorded in farm 4. The salinity (ppt) levels in farm 1 and 2 were in the range of 9.0 to 16.0 and farm 3 had salinity levels in the range of 25.0–26.0 ppt. Mud crab is a euryhaline animal which can tolerate water salinities

ranging from 5 to 33.2 ppt (Jia and Chen, 2001). Juvenile crabs can survive in lower salinities (upto 5 ppt) than larvae. Adult crabs are more tolerant to salinity variation.

Table 2. Hydrographic parameters of the four crab farms selected for the study

Source of sample	Surface temp. (range) °C	pH (range)	Salinity (range) ppt
Farm 1	30-31	6.0- 7.0	9.0 -11.0
Farm 2	30-31	6.6- 7.0	12.0 -16.0
Farm 3	30-31	6.6 - 6.9	25.0 - 26.4
Farm 4	29-30	6.8 - 7.0	4.0 - 5.0

Microbial counts

The aerobic mesophilic counts of water samples from the four farms ranged from 3.28 to 4.45 log₁₀ cfu g⁻¹ and in the mud samples were in the range of 4.96 to 6.96 log₁₀ cfu g⁻¹ (Tables 3 and 4). The mesophilic counts on fresh whole crab were in the range of 5.2- 6.45 log₁₀ cfu g⁻¹ and in claw meat it ranged from 3.99 to 5.45 log₁₀ cfu g⁻¹. The

Table 3. Mean total aerobic bacterial count of water and mud from four crab (*S. serrata*) farms located in Kerala, India

Bacteriological parameters		Mean (± SD) bacterial count (log ₁₀ cfu g ⁻¹)			
		Farm 1	Farm 2	Farm 3	Farm 4
Pond water					
Total aerobic bacterial count	37 °C	4.29±0.132	5.43±0.103	3.28±0.056	4.22±0.021
	30 °C	4.36±0.128	5.45±0.073	3.31±0.035	4.23±0.007
	20 °C	3.2±0.122	3.78	3.06±0.019	3.00±0.055
	7 °C	2.78±0.007	3.37±0.006	1.98±0.199	2.8±0.099
Pond sediment					
Total aerobic bacterial count	37 °C	4.99±0.092	6.26±0.059	5.21±0.186	6.89±0.016
	30 °C	4.96±0.033	6.3±0.032	5.39±0.07	6.96±0.007
	20 °C	4.04±0.039	5.93±0.003	4.96±0.017	5.93±0.02
	7 °C	3.57±0.088	4.15±0.076	3.69±0.021	4.89±0.012

Table 4. Mean total aerobic bacterial count in farmed crab (*S. serrata*) from four farms located in Kerala, India

Bacteriological parameters		Mean (±SD) bacterial count (log ₁₀ cfu g ⁻¹)			
		Farm 1	Farm 2	Farm 3	Farm 4
Crab whole					
Total aerobic bacterial count	37 °C	5.47± 0.026 ^a	6.45± 0.045	5.24± 0.102	5.52± 0.033
	30 °C	5.51± 0.012	6.43±0.047	5.25±0.045	6.09±0.08
	20 °C	4.49±0.014	6.09±0.031	5.14±0.034	5.40±0.029
	7 °C	3.84± 0.057	4.03±0.008	3.57±0.041	4.94±0.046
Total <i>Vibriosis</i>		3.14±0.047	4.73± 0.048	4.58± 0.027	3.19±0.031
Crab claw meat					
Total aerobic bacterial count	37 °C	5.42±0.008	4.67±0.094	4.16±0.011	4.77±0.064
	30 °C	5.45±0.017	4.71±0.026	3.99±0.031	4.91±0.019
	20 °C	4.49±0.007	3.84±0.006	3.99±0.002	4.71±0.037
	7 °C	2.44±0.062	2.55±0.068	2.66±0.184	4.58±0.176
Total <i>Vibriosis</i>		2.13±0.046	2.93±0.051	2.02±0.021	3.15±0.107

mesophilic counts of water and crab samples from farm 2 were significantly higher than that from farms 1, 3 and 4 ($p < 0.001$).

Aerobic mesophilic counts at 37 °C and 30 °C for water, sediment and crab did not differ significantly. Significant differences ($p < 0.05$) were noticed between aerobic counts at 20 °C and mesophilic counts. The mean mesophilic count of $5.67 \log_{10} \text{ cfu g}^{-1}$ in fresh whole crab indicates acceptable crab quality. According to International Commission of Microbiological Specification for Foods (ICMSF, 1998), counts of chilled / frozen crab meat below 10^5 cfu g^{-1} are considered good quality and counts between 10^5 and 10^6 cfu g^{-1} are considered marginally acceptable quality. The results indicate that any delay in icing after harvest will favour the multiplication of these mesophilic flora and lower the quality of crab. The mesophilic bacterial load in the range of $3.4 \times 10^4 - 7.2 \times 10^5 \text{ cfu g}^{-1}$ were reported in muscle of crab (*S. serrata*) caught off Cochin (George and Gopakumar, 1988). They have reported increase in bacterial load to 10^7 cfu g^{-1} on storage of crab muscle at ambient temperature for 6 h. The mesophilic counts of farmed crab *S. serrata* fall within this range. Studies on the microbiological characteristics of sixteen samples of dungeness crab (*C. magister*) reared in Oregon, USA have shown that the mesophilic counts were in the range of 10^4 to 10^7 with the geometric mean of 10^5 cfu g^{-1} (Lee and Pfeifer (1975). Reinhard *et al.* (1996) reported aerobic plate counts in the range of 7.4×10^3 to 4.6×10^8 in fresh picked crab meat from twelve different blue crab processing facilities in Virginia, USA.

The psychrotrophic counts (7 °C) on whole crab ranged from $3.84 - 4.94 \log_{10} \text{ cfu g}^{-1}$ and in claw meat, the count was in the range of 2.44 to $4.58 \log_{10} \text{ cfu g}^{-1}$. Psychrotrophic counts of water, sediment and crab samples were significantly lower than those at 30 °C ($p < 0.01$) indicating that a minor fraction (<5%) of the total microflora of crab and farm environment is to be categorised as psychrotrophic organisms. The psychrotrophic counts in whole crab from farm 4 was high ($4.9 \log_{10} \text{ cfu g}^{-1}$). This

could be attributed to the variations in the percentage composition of various bacterial genera in crab from various farms. The results suggest that crabs should be washed immediately after harvest and should be iced and processed immediately to eliminate quality loss.

Vibrio population in *S. serrata* constituted less than 5% of the aerobic mesophilic flora enumerated at 37 °C (Table 4). In farms 1 and 4, the count was around $3 \log_{10} \text{ cfu g}^{-1}$ where as in farm 2 and 3, it was around $4.5 \log_{10} \text{ cfu g}^{-1}$. *Vibrios* are halophilic and therefore, this difference in count could be attributed to the difference in salinity of the water.

Bacterial flora of farmed *S. serrata*

The aerobic mesophilic flora of mud crab was dominated by gram-negative bacteria belonging to genera *Vibrio*, *Moraxella*, *Acinetobacter* and *Pseudomonas/Shewanella* and family Enterobacteriaceae. Among Gram-positive bacteria, *Micrococcus*, *Staphylococcus* and *Bacillus* were isolated to a lesser extent. The psychrotrophic flora was dominated by *Pseudomonas/Shewanella*, *Aeromonas*, *Moraxella*, *Acinetobacter* and *Flavobacterium/Cytophaga*. The percentage composition of various genera in farm water and crab meat varied between farms (Table 5 and 6). The diversity of bacterial species associated with the tissues of crab was greatest for the samples collected from farm 4. Bacteria of Enterobacteriaceae family, particularly *Klebsiella pneumoniae*, *Citrobacter* and *Enterobacter* were recovered from crab meat. The isolation frequency of *Vibrio* in crab meat varied from 12-35.71% and the flora comprised of *V. harveyi*, *V. holisae*, *V. fisheri*, *V. fluvialis*, *V. ordalii*, *V. anguillarum* and *V. mimicus*. Among Aeromonadaceae, *Aeromonas veronii* biovar *sobria*, *A. caviae* and *A. jandaei* were recovered. The results of the study agree well with that of Faghri *et al.* (1984) who reported abundance of *Acinetobacter*, *Moraxella*, *Vibrio* and *Achromobacter* species in dungeness crabs collected off the mouth of the Columbia River in USA. In the same study, they reported dominance of *Pseudomonas* species in rock crabs collected off Maine and other

Table 5. Percentage composition of the aerobic mesophilic bacteria in water from four crab farms located in Kerala, India

Bacterial species	Percentage composition of the mesophilic flora			
	Farm 1	Farm 2	Farm 3	Farm 4
<i>Vibrio</i>	30	25	27.3	10
<i>Aeromonas</i>		8.3		20
Enterobacteriaceae	10	25		10
<i>Pseudomonas/Shewanella</i>	30	16.7	36.3	10
<i>Acinetobacter</i>	20			10
<i>Bacillus</i>			18.2	20
<i>Micrococcus</i>	10	8.3	18.2	
<i>Flavobacterium/Cytophaga</i>	16.7		20	

important isolates were *Acinetobacter*, *Moraxella*, *Vibrio*, *Klebsiella*, *Citrobacter* and *Achromobacter*. Lee and Pfeifer, (1975) reported growth of *Moraxella*, *Pseudomonas*, *Acinetobacter* and *Flavobacterium/Cytophaga* in dunginess crab at refrigeration temperatures.

Bacteria belonging to the genera *Vibrio*, *Aeromonas*, *Pseudomonas*, *Alteromonas*, *Flavobacterium*, *Spirillum*, *Moraxella*, *Pasteurella* and *Photobacterium* are all reported as probable agents involved in shell disease syndrome in crustaceans (Getchell, 1989). Bacteriological investigation of shell disease in the deep sea red crab, *Geryon quinquedens*, has revealed the presence of *Vibrio* and *Flavobacterium* spp. and *Escherichia coli* in lesions (Bullis *et al.*, 1988). *Vibrio* spp. and aeromonads were primarily responsible for progressive infections in blue crab, *Callinectes sapidus* Rathbun and were the predominant bacterial type in heavily infected crabs with shell disease (Davis and Sizemore, 1982; Welsh and Sizemore, 1985; Noga *et al.*, 2000; Vogan *et al.*, 2002). *V. fluvialis*, *V. hollisae* and *V. mimicus* accounted for gastroenteritis associated infections in humans due to consumption of contaminated

raw or undercooked shellfish such as oysters, clams, mussels, or crabs (Huss, 1994; Morris, 1999). *A. hydrophila*, *A. sobria* and *A. caviae* have been described as emergent food-borne pathogen of increasing importance causing gastroenteritis (Kirov, 1997). Potentially, *Aeromonas* spp. can become a serious food problem as many of them can grow at refrigeration temperatures. *A. hydrophila* is also often found in association with disease outbreaks in aquaculture production (Nielsen *et al.*, 2001). Fang *et al.* (2008) and Li *et al.* (2005) reported pathogens such as *A. veronii*, *V. mimicus*, *V. parahemolyticus*, *Aeromonas trota* and *A. hydrophila* in crab culture. Handling and cross contamination might be a health hazard, particularly with susceptible populations.

Indicator and pathogenic bacteria in mud crab

Faecal coliforms and *Escherichia coli* were recovered from crab and water from all the four farms (Tables 7 and 8). *E. coli* counts in crab meat from farm 1 and 3 were within acceptable limit (11 g^{-1}) and that from farm 4 exceeded the maximum limit (500 g^{-1}) for acceptability of crab meat, as recommended by the ICMSF (1998).

Table 6. Percentage composition of the aerobic mesophilic bacteria in mud crab from four farms located in Kerala, India

Bacterial genera/group	Percentage composition of the mesophilic flora			
	Farm 1	Farm 2	Farm 3	Farm 4
<i>Vibrio</i>	35.71	13.3	26.6	12
<i>Aeromonas</i>	7.14	6.7	6.6	8
Enterobacteriaceae	14.29	20		12
<i>Pseudomonas/Shewanella</i>	14.29	13.3	20	24
<i>Acinetobacter</i>	7.14	13.3	6.7	4
<i>Moraxella</i>	14.29	6.7	20	12
<i>Bacillus</i>		13.3	6.7	4
<i>Micrococcus</i>	7.14	6.7	6.7	8
<i>Flavobacterium/Cytophaga</i>		6.7	6.7	12
<i>Staphylococcus</i>				4

Table 7. Indicator bacterial counts in water and sediment from four crab farms located in Kerala, India

Bacteriological parameters	Mean bacterial count ($\log_{10} \text{cfu g}^{-1}$)			
	Farm 1	Farm 2	Farm 3	Farm 4
Water				
Faecal coliforms*	2.41+0.239**	3.54+0.5	1.85+0.194	1.53+ 0.127
<i>Escherichia coli</i> *	1.91+0.261	3.54+0.5	1.30+0.349	0.67+0.72
<i>Staphylococcus aureus</i>	1.24+0.238	ND	ND	ND
Faecal Streptococci	1.67+0.37	2.13+0.173	ND	1.52+0.085
<i>Clostridium perfringens</i> *	ND	0.28+0.323	ND	ND
Sediment				
Faecal coliforms*	1.80+0.151	0.45+0.5	-0.05	1.85+0.194
<i>Escherichia coli</i> *	ND	-0.044	-0.044	1.53+0.128
<i>Staphylococcus aureus</i>	0.65+0.65	ND	ND	ND
Faecal Streptococci	2.45+0.151	1.45+0.151	1.39+0.088	1.39+0.088
<i>Clostridium perfringens</i> *	1.22+0.261	0.45+0.5	1.04+0.438	1.26+0.218

* \log_{10} MPN g^{-1} , ** standard deviation; ND-Not Detected

Enterococci counts were significantly high in crab meat than that in water ($p < 0.05$). The levels of faecal coliforms, Enterococci, *Clostridium perfringens*, and *E. coli* in the water collected from farm 2 were very high (Table 7) compared to that of farms 1, 3 and 4. The crabs collected from farm 2 and 4 had significantly high levels ($p < 0.05$) of faecal coliforms and Enterococci (Table 8). *C. perfringens* was not detected in crab from farm 2. However, water samples were positive for *C. perfringens*. *Staphylococcus aureus* could not be detected in water and in crab samples from farm 4. *S. aureus* counts were within the acceptable limit (10^3 cfu g^{-1}) recommended by ICMSF (1998). These microbial groups are important in foods as indicators of hygienic quality of foods.

The study revealed presence of pathogenic bacteria such as *V. fluvialis*, *V. hollisae*, *V. mimicus*, *A. veronii* biovar *sobria*, *A. caviae*, *A. jandaei* and high numbers of faecal coliform as well as enterococci in farmed mud crab. This knowledge will increase our understanding of the effects of aquaculture operations on bacterial community composition in the crab and provide necessary data for the development of control measures in crab farms. The presence of emerging bacterial pathogens, such as *Aeromonas* and *Vibrio* spp., in the crab farms is of great importance from both epidemiological and ecological points of view. The results of the study indicate that good farming and post-harvest practices should be adopted to improve the microbiological quality of farmed crab.

Table 8. Indicator bacterial counts in mud crab *Scylla serrata* from four farms located in Kerala, India

Bacteriological parameters	Mean bacterial count (\log_{10} cfu g^{-1})			
	Farm 1	Farm 2	Farm 3	Farm 4
Whole crab				
Faecal coliforms*	0.85+0.194**	2.35+0.305	1.07+0.111	2.85+0.194
<i>Escherichia coli</i> *	0.45+0.5	1.35+0.306	0.30+0.35	2.72+0.321
<i>Staphylococcus aureus</i>	1.66+ 0.184	1.59+0.181	0.801+0.80	ND
Faecal Streptococci	3.10+0.199	3.67+0.376	1.75+0.15	2.66+0.052
<i>Clostridium perfringens</i> *	0.07+0.11	ND	0.79+0.19	0.79+0.19
Crab claw meat				
Faecal coliforms*	2.35+0.306	1.89+0.246	0.75+0.096	2.99+0.044
<i>Escherichia coli</i> *	2.30+0.349	0.78+0.176	0.07+0.11	2.35+0.305
<i>Staphylococcus aureus</i>	1.86+0.256	1.63+0.151	1.24+0.239	2.04+0.039
Faecal Streptococci	2.30+0.022	3.68+0.059	1.24+0.239	2.06+0.057
<i>Clostridium perfringens</i> *	0.39+0.212	ND	-0.39	ND

* \log_{10} MPN g^{-1} , ** standard deviation, ND-Not Detected

The high coliform and enterococci levels in farmed crab and water indicates that rearing practices such as feeding and pond fertilization could have influenced the microflora. Buras *et al.* (1987) have shown that bacterial levels of pond raised fish may increase considerably in edible tissues after a threshold level has been surpassed in the environment. While analysing fresh picked crab meat from twelve different blue crab processing facilities, Reinhard *et al.* (1996) observed coliform and fecal coliform counts in the range of <0.3 to 32.8 MPN g^{-1} and <0.3 to 2.26 MPN g^{-1} , respectively and *Escherichia coli* counts ranged from <0.3 to 0.77 MPN g^{-1} . The increase in antimicrobial resistance in enterococci is an issue of increasing concern in itself and also because enterococci can transfer antibiotic resistance genes to other pathogens (Bonadio *et al.*, 2000). When present in high levels in ready to eat foods, *Enterococcus* species may present a health risk to consumers (Franz *et al.*, 1999).

The present study demonstrated differences in the bacterial composition of crabs from different farming

systems. Increased knowledge about the environmental conditions that lead to disease, as well as about the interactions of these pathogens with other microbial species, could help in the development of management strategies in crab farms.

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References

- Alsina, M. and Blanch, A. R. 1994. A set of keys for biochemical identification of *Vibrio* species. *J. Appl. Bacteriol.*, 76: 79-85.
- APHA 1998. *Standard methods for the examination of water and waste water*, 20th edn., American Public Health Association, Inc., Washington D. C. Part 9000-9221, p. 48-59.
- Austin, B. 1988. Identification. In: Austin, B. (Ed.), *Methods in Aquatic Bacteriology*. John Wiley & Sons Ltd., London, p. 95 - 112.

- Bolinches, J., Romalde, J. L. and Toranzo, A. E. 1988. Evaluation of selective media for isolation and enumeration of *Vibrios* from estuarine waters. *J. Microbiol Meth.*, 8: 151–160.
- Bonadio, M., Meini, M., Tagliaferri, E., Gigli, C. and Vigna, A. 2000. Enterococcal glycopeptide resistance at an Italian teaching hospital. *J. Antimicrob. Chemother.*, 46: 129-131.
- Bullis, R., Leibovitz, L., Swanson, L. and Young, R. 1988. Bacteriological investigation of shell disease in the deep-sea red crab, *Geryon quinquedens*. *Biol Bull.*, 175: 304.
- Buras, N., Duek, L., Niv, S., Hefner, B. and Sandbank, E. 1987. Microbiological aspects of fish grown in treated waste water. *Water Res.*, 21: 1-10.
- Cockney, R. R. and Chai, T. J. 1991. Microbiology of crustacea processing crab. In: Ward, D. R. and Hackney, C. W. (Eds.), *Microbiology of marine food products*, Van Nostrand Reinhold, New York. p. 41-63.
- Davis, J. W. and Sizemore, R. K. 1982. Incidence of *Vibrio* species associated with blue crabs (*Callinectes sapidus*) collected from Galveston Bay, Texas. *Appl. Environ. Microbiol.*, 43(5): 1092–1097.
- Elliot, E. L., Kaysner, C. A., Jackson, L. and Tamplin, M. L. 1998. *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus* and other *Vibrio* species. In: *Food and Drug Administration Bacteriological Manual*, 8th edn. (Revision A), AOAC International, Gaithersburg, MD.
- Faghri, M. A., Pennington, C. L., Cronholm, L. S. and Atlas, R. M. 1984. Bacteria associated with crabs from cold waters with emphasis on the occurrence of potential human pathogens. *Appl. Environ. Microbiol.*, 47: 1054-1061.
- Fang, H., Chen, C. Z., Zhang, X. J., Gong, Y. F. and Ge, M. X. 2008. Examination of the pathogenic *Aeromonas veronii* isolated from crab *Eriocheir sinensis*. *Chin. J. Zoonoses*, 24 (1): 45–49.
- FDA (Food and Drug Administration) 1998. *FDA Bacteriological analytical manual*, 8th edn., AOAC International, Gaithersburg, MD.
- Franz, C. M. A. P., Holzapfel, W. H. and Stiles, M. E. 1999. Enterococci at the cross roads of food safety. *Int. J. Food Microbiol.*, 47: 1-2.
- George, C. and Gopakumar, K. 1988. Spoilage changes in the muscle of crab, *Scylla serrata* stored at three different temperatures. In: Mohan Joseph, M. (Ed.), *The First Indian Fisheries Forum Proceedings*. Asian Fisheries Society, Indian Branch, Mangalore, p. 347-349.
- Getchell, R. G. 1989. Bacterial shell disease in crustaceans: a review. *J. Shellfish Res.*, 8: 1-6.
- Huss, H. H. 1994. *Assurance of seafood quality*. FAO Fisheries Technical Paper 334, FAO, Rome.
- ICMSF (International Commission on Microbiological Specifications for Foods) 1998. *Microorganisms in Foods, Microbial Ecology of Food Commodities*. Baltimore: Blackie Academic & Professional.
- Jia, J. and Chen, J. 2001. Mud crab biology and culture. In: *Sea farming and sea ranching in China*. FAO Fisheries Technical Paper, FAO, Rome. 418: 75 pp.
- Kirov, S. M. 1997. *Aeromonas* and *Plesiomonas* species. In: Doyle, M., Beuchat, L. and Montville, T. (Eds.), *Food microbiology: fundamentals and frontiers*. ASM Press, Washington, D. C., p. 265-287.
- Krieg, N. R. and Holt, J. G. 1984. *Bergey's manual of systematic bacteriology*. Vol. 1, Williams and Wilkins, Baltimore, USA.
- Lalitha, K. V. and Surendran, P. K. 2004. Bacterial microflora associated with farmed freshwater prawn, *Macrobrachium rosenbergii* (de Mann) and the aquaculture environment. *Aquacult. Res.*, 35: 1-7.
- Lee, J. S. and Pfeifer, D. K. 1975. Microbiological characteristics of dungeness crab (*Cancer magister*) *Appl. Microbiol.*, 30: 72-78.
- Li, J. N., Li, Y. Y., Hu, S. K., Li, L., Fang, B., Yu, W. Y. and Zhang, X. H. 2005. Analysis on pathogen producing ascetic fluid disease of *Eriocheir sinensis*. *J. Fish. Sci. China*, 12 (3): 267–274.
- Loaharanu, P. and Lopez, A. 1970. Bacteriological and shelf-life characteristics of canned, pasteurized crab cake mix. *Appl. Microbiol.*, 19: 734-741.
- Maugeri, T. L., Caccamo, D. and Gugliandolo, C. 2000. Potentially pathogenic vibrios in brackishwaters and mussels. *J. Appl. Microbiol.*, 89: 261- 266.
- Morris, J. G. 1999. *Vibrios* on the half-shell. *Culture*, 20: 5–8.
- Nielsen, M. E., Hoi, I., Schmidt, A. S., Qian, D., Shimata, T., Shen, J. Y. and Larsen, J. L. 2001. Is *Aeromonas hydrophila* the dominant motile *Aeromonas* species that cause disease outbreaks in aquaculture production in the Zhejiang Province of China?, *Dis. Aquat. Organ.*, 22: 23–29.
- Noga, E. J., Smolowitz, R. and Khoo, L. H. 2000. Pathology of shell disease in the blue crab, *Callinectes sapidus* Rathbun, (Decapoda: Portunidae). *J. Fish Dis.*, 23: 389–399.
- Paterson, B. D. 2009. Advances in the culture of crabs. In: Burnell, G. and Allan, G. (Eds.) *New technologies in Aquaculture: Improving production efficiency, quality and environmental management*. Woodhead Publishing Limited, Cambridge, CB22 3HJ, UK, p. 845-865.
- Pfeffer, C. and Oliver, J. D. 2003. A comparison of thiosulphate-citrate-bile salts-sucrose (TCBS) agar and thiosulphate-chloride-iodide (TCI) agar for the isolation of *Vibrio* species from estuarine environments. *Let. Appl. Microbiol.*, 36: 150–151.
- Reinhard, R. G., Mc Adams, T. J., Flick, G. J., Wittman, R. F., Croonenberghs, R. E. and Diallo, A. A. 1996. Qualitative and quantitative analysis of *Campylobacter jejuni* and *Campylobacter coli* in fresh blue crab (*Callinectes sapidus*) meat. *J. Aqua. Food Product. Technol.*, 4: 31 – 36.

- Sneath, P. H. A., Mair, N. S., Sharpe, M. E. and Holt, J. G. 1986. *Bergey's manual of systematic bacteriology*, Vol. 2, Williams and Wilkins, Baltimore, USA.
- Vogan, C. L., Costa-Ramos, C. and Rowley, A. F. 2002. Shell disease syndrome in the edible crab *Cancer pagurus*-isolation, characterization and pathogenicity of chitinolytic bacteria. *Microbiol.*, 148: 743-754.
- Welsh, P. C. and Sizemore, R. K. 1985. Incidence of bacteremia in stressed and unstressed populations of the blue crab, *Callinectes sapidus*. *Appl. Environ. Microbiol.*, 50: 420-425.
- West, P. A. 1989. Human pathogens and public health indicator organisms in shellfish. In: Austin, B. and Austin, D. A. (Eds.), *Methods for the microbiological examination of fish and shellfish*, Halsted Press, Chichester, p. 273-308.